REMARKS

Claims 17 to 33 are pending with claims 17 to 23 and claim 33 canceled herein. Claims 27 to 32 have been withdrawn from consideration, and new claims 34 to 38 have been added. Claims 24 to 26 and 34 to 38 are presently under examination.

Regarding the claim amendments and new claims

Claims 24 to 26 have been amended herein to replace "substantially pure" ARP3 polypeptide with "isolated" ARP3 polypeptide. The amendment is supported throughout the specification, for example, at page 62, lines 17-26, which discloses that isolated ARP polypeptides are useful, and at page 65, lines 19-24, which indicates that purified ARP polypeptides can be useful for producing antibodies.

Claim 24 further has been amended herein to more clearly indicate that an isolated ARP3 polypeptide of the invention includes an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5. New claim 24 is supported throughout the specification, for example, at page 12, lines 6-7, and Figure 3, which shows the amino acid sequence (SEQ ID NO:5) of ARP3.

Claim 26 has been amended herein to indicate that the claimed isolated ARP3 polypeptide fragment includes "at least ten" contiguous amino acids of SEQ ID NO:5. The amendment to claim 26 is supported throughout the specification, for example, at page 23, line 30, to page 24, line 8, which indicates that a

fragment can include 9, 10, 11, 12 or more contiguous amino acids of the amino acid sequence shown as SEQ ID NO:5, 7 or 9.

New claims 34 to 37 are directed to an isolated ARP3 polypeptide which includes an amino acid sequence having at least 55%, 65%, 75% or 85% amino acid identity, respectively, with residues 1 to 537 of SEQ ID NO:5. New claims 34 to 37 are supported throughout the specification, for example, at page 24, lines 12-16, which indicates that an ARP3 polypeptide can have 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to human ARP3 (SEQ ID NO:5).

New claim 38 is directed to an isolated ARP3 polypeptide containing the amino acid sequence shown as SEQ ID NO:5 with substitution of one or more conservative amino acid residues. New claim 38 is supported throughout the specification, for example, at page 27, lines 3-10, which discloses modifications to SEQ ID NOS:5, 7 and 9 such as an addition, deletion or substitution of one or more amino acid residues.

The amendments and new claims are supported by the specification as filed and do not add new matter. Applicant therefore respectfully requests that the Examiner enter the amendments and new claims.

Regarding the references filed with the Information Disclosure Statement

The present Office Action indicates that the references accompanying the form PTO-1449 of June 25, 2001, are missing from the PTO file. As a courtesy, Applicant provides herewith a second copy of the 39 references filed with the Information Disclosure Statement of June 25, 2001.

Applicant further submits herewith as Exhibit A a copy of the postcard stamped by the Patent and Trademark indicating that, on June 25, 2001, an Information Disclosure Statement and PTO Form-1449 with 39 references were received by the PTO. Applicant respectfully reminds the Examiner that, should any new rejections be issued on the basis of the references disclosed in the PTO-1449 received on June 25, 2001, an Office Action containing the new rejections may not be made final.

Regarding the Rejections under 35 U.S.C. § 112, second paragraph

The rejection of claims 24 to 26 under 35 U.S.C. § 112, second paragraph, as allegedly vague and indefinite due to the term "substantially pure" is respectfully traversed. In making the rejection, the Examiner alleges that this is a relative term which renders the claims indefinite.

Applicant submits that the claims are clear and definite as written in view of the specification. In particular, it is clear to the skilled person that the term "substantially pure" refers to a highly purified polypeptide preparation, which is readily distinguished, for example, from a cell extract or fraction in which the polypeptide may be present.

While Applicant maintains that the claims are clear and definite as written, claims 24 to 26 have been amended herein in order to further prosecution of the subject application to replacing the term "substantially purified" with "isolated." In view of the above remarks and amendments, Applicant respectfully requests that the Examiner reconsider and remove the rejection of claims 24 to 26 under 35 U.S.C. § 112, second paragraph.

Regarding the Rejection under 35 U.S.C. §101

Applicant respectfully traverses the rejection of claims 24 to 26 under 35 U.S.C. §101 as allegedly lacking a specific asserted utility or a well established utility. As acknowledged in the Office Action, the specification discloses that the ARP3 polypeptide SEQ ID NO:5 is a predicted polypeptide encoded by SEQ ID NO:4, a nucleic acid isolated from mRNA of the prostate carcinoma cell line LNCaP following upregulation by androgen. The specification discloses making antibodies

specific for the ARP3 polypeptide SEQ ID NO:5 as well as diagnostic and therapeutic uses for such antibodies.

The Examiner specifies several grounds for the utility rejection, as follows:

1) Although ARP3 RNA (SEQ ID NO:4) is expressed in the LNCaP cell line and is androgen regulated, the Examiner asserts that it is unpredictable whether the ARP3 polypeptide exists in nature.

2) The ARP3-encoding nucleic acid sequence SEQ ID NO:4 was obtained from the LNCaP cell line, and such a cell line does not necessarily have the same characteristics nor duplicate the complex conditions of a tumor *in vivo*.

3) There are no data showing that the nucleic acid sequence SEQ ID NO:4 or the predicted encoded polypeptide SEQ ID NO:5 and its variants are over-expressed in prostate cancer tissues as compared to normal prostate tissue.

4) Even if the claimed ARP3 polypeptide were expressed in a prostate-specific manner, this prostate specificity does not constitute a specific utility since such a utility is shared by several other prostate specific polypeptides.

5) Utility for the ARP3 polypeptide is questionable because neither the specification nor any art of record teaches the function of the ARP3 polypeptide SEQ ID NO:5.

Applicant maintains that use of an ARP3 polypeptide to generate antibodies reactive to ARP3 is a specific, credible and

substantial utility. Such anti-ARP3 antibodies can be used, for example, as imaging reagents to detect prostate cancer metastases. In this regard, the specification teaches that a binding agent which selectively binds ARP3, ARP4 or ARP5, such as a monoclonal antibody, can be useful in imaging techniques to detect neoplastic prostate cells and image "secondary sites of metastasis" (specification at page 57, line 16, to page 58, line 2). This utility is further corroborated by the data presented in the accompanying Rule 132 Declaration demonstrating that ARP3 is upregulated in a variety of biopsy specimens from metastases secondary to prostate cancer as compared to localized prostate cancer. These results, which are summarized in Table 1 and Figure 1 of the attached Rule 132 Declaration, corroborate the utility of an anti-ARP3 antibody as an imaging agent for detecting secondary sites of prostate cancer metastasis.

1. In regard to the first specific point raised by the Examiner in regard to the utility rejection, Applicant submits that an ARP3 polypeptide exists in nature, as taught in the specification and corroborated by additional data. Specifically, the amino acid sequence of the ARP3 polypeptide encoded by the androgen-inducible ARP3 cDNA is shown in Figure 3 of the specification (see, also, page 74, lines 20-22). As corroboration that an ARP3 polypeptide is expressed in nature as taught in the specification, Applicant submits herewith as Exhibit B, Ansley et al., "Basal body dysfunction is a likely cause of pleiotrophic Bardet-Biedl syndrome," Nature 425:628-633 (2003). Ansley et al. report protein expression of BBS8, a

recently cloned polypeptide with an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5. In particular, the amino acid sequence of BBS8 corresponds to the amino acid sequence of human ARP3 (SEQ ID NO:5) from residue 33 to residue 537, as illustrated in the alignment presented in Exhibit C.

As shown in the Ansley et al. Nature paper attached as Exhibit B, the BBS8 polypeptide was expressed in nature in ciliated structures, as demonstrated by staining of tissue sections with polyclonal antisera to human BBS8 (page 631, first column, second complete paragraph; page 630, Figure 3). For example, in Figure 3, panel A, Ansley et al. show expression of BBS8 protein in maturing spermatids, and in panel I, Ansley et al. show specific reactivity of anti-BBS8 antiserum with a Myc-BBS8 construct expressed in HEK293 cells as compared to pre-immune serum. Thus, as taught in the specification and further corroborated by subsequent data, an ARP3 polypeptide having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5 exists in nature.

2. As outlined above, the utility rejection is further based, in part, on the assertion that because the ARP3-encoding nucleic acid sequence SEQ ID NO:4 was obtained from a tumor cell line (LNCaP), this gene is not necessarily an *in vivo* marker for prostate tissue or tumors.

As discussed above, Applicant submits in the accompanying Rule 132 Declaration data showing ARP3 expression in biopsy specimens from various metastasized prostate cancers. The data shown in Figure 1 and summarized in Table 1 of the

attached Declaration evidence the relevance of ARP3 to prostate cancer *in vivo*. Thus, these data corroborate the *in vivo* utility of ARP3.

3. The Office Action asserts that ARP3 and its variants may not be over-expressed in prostate cancer tissues as compared to normal prostate tissue, and queries whether ARP3 or its variants are expressed outside of the prostate.

Firstly, low level expression of the ARP3 polypeptide in normal prostate is evidenced by the northern blot analysis shown in Figure 2 of the attached Declaration. In particular, the most abundant expression of ARP3 was observed in testis and ovary, while little ARP3 expression was present in normal prostate and other tissues. One skilled in the art readily extrapolates the absence of expression at the RNA level to the absence of ARP3 protein expression. Furthermore, differential expression of ARP3 RNA in metastatic prostate cancer specimens as compared to localized prostate cancer specimens, shown in the attached Rule 132 Declaration and discussed above, substantiates the utility of an anti-ARP3 antibody, for example, as a diagnostic or imaging agent for metastatic prostate cancer.

4. The Office Action further states that, even if the claimed ARP3 polypeptide were expressed in a prostate-specific manner, such prostate specificity does not constitute a specific utility since such a utility would be shared by several other prostate specific polypeptides.

Firstly, in contrast to the Examiner's assertion, a "specific utility" need not be unique to a single polypeptide or

other molecule. Certainly, there is nothing in the patent statutes or utility guidelines specifying that a utility may not be common to two or more different molecules. Indeed, multiple patents have clearly issued on two or more different molecules, each useful, for example, as anti-cancer therapeutics or anti-inflammatory therapeutics, evidencing that utilities need not be unique. Thus, even if another marker had exactly the same expression profile as ARP3, anti-ARP3 antibodies nevertheless have utility, for example, as imaging reagents for detecting metastases secondary to prostate cancer.

Secondly, the skilled artisan understands, as taught in the specification, that the potential of novel tumor markers can be optimized by utilizing them in combination with other tumor markers (page 2, lines 16-19) and that an ARP polypeptide can be used in combination with other molecules as a specific marker for prostate cells or prostate neoplastic conditions (page 13, lines 15-21). As an example, the specification teaches that ARP polypeptide or nucleic acid expression can be used in conjunction with other prostate markers such as prostate specific antigen (PSA), human glandular kallikrein 2 and prostase/PRSS18 for diagnostic or therapeutic applications (page 52, lines 1-17). In sum, it is well known to those skilled in the art that combinations of markers including multiple imaging reagents can improve, for example, diagnostic sensitivity or specificity. Thus, although not required, an ARP3 antibody likely has a utility distinct from that of most or all other known antibodies, for example, antibodies against prostate specific antigen (PSA). Thus, this ground for rejection is rendered moot.

5. The Office Action additionally indicates that the utility requirement has not been met because no function for the ARP3 polypeptide is taught.

Applicant submits that, to meet the utility requirement, a specific, credible and substantial utility must be present. However, there is no requirement to teach the mechanism or 'how' a molecule works. As held in *In re Cortwright,* 49 U.S.P.Q.2d. 1646 (Fed. Cir. 1999), § 101 contains no requirement that an inventor know or state how the invention works.

Furthermore, it is well established that useful diagnostic markers for a disease or a condition need not be involved in the etiology of a disease and need not have a known function. Such markers are useful diagnostic or imaging tools if the expression of these markers is associated with a particular disease or condition, irregardless of their function or any role in the disease. In the present case, ARP3 expression is associated with metastatic prostate cancer, indicating that an anti-ARP3 antibody can be useful as an imaging agent to detect prostate cancer metastases irregardless of a known or unknown function of ARP3. Applicant therefore submits that the utility requirement has been met irregardless of whether a function for ARP3 or functional role in cancer has been disclosed.

In view of the above remarks, Applicant respectfully requests that the Examiner reconsider and remove the rejection of claims 24 to 26 as allegedly lacking utility under 35 U.S.C. § 101.

Regarding the Written Description Rejection under

35 U.S.C. § 112, first paragraph

Applicant respectfully traverses the rejection of claims 24 to 26 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor had possession of the claimed invention at the time the application was filed. In making the rejection, the Examiner asserts that an ARP3 polypeptide containing an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5 encompasses variants having conservative and other substitutions of SEQ ID NO:5 and further asserts that "an" amino acid sequence can be a two amino acid fragment of SEQ ID NO:5.

Applicant submits that the specification provides written description sufficient to reasonably convey to one skilled in the art that Applicant had possession of the invention at the time the application was filed. In this regard, the specification discloses isolation of the human ARP3 cDNA (Example I), providing SEQ ID NO:5 as an exemplary ARP3 polypeptide. Additional written description is provided for the ARP3 polypeptides of the invention, for example, at page 24, lines 12-16, which indicates that an ARP3 polypeptide has 45% or more amino acid sequence identity to SEQ ID NO:5, and at page 24, lines 27-30, which indicates that an ARP3 polypeptide can be, for example, a species homolog such as a mammalian or non-mammalian homolog of human ARP3. In view of the above, claim 24, directed to an ARP3 polypeptide containing an amino acid

sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5, clearly encompasses polypeptides with sequence variations as compared to SEQ ID NO:5. These sequence variations are precisely defined by reference to SEQ ID NO: 5 and the recitation of at least 45% amino acid identity to the specified sequence. In short, in view of the specification, one skilled in the art appreciates that the inventor had possession of the invention at the time the application was filed.

Regarding a two amino acid fragment of SEQ ID NO:5

The Office Action further asserts that claim 24 encompasses unrelated sequences that are two amino acid fragments of SEQ ID NO:5.

Applicant respectfully submits that an unrelated sequence sharing only two amino acids with SEQ ID NO:5 is distinct from the claimed ARP3 polypeptides and fragments of the invention. In particular, a two amino acid sequence does not have "at least 45% amino acid identity" with SEQ ID NO:5, a sequence of more than 500 residues (specification at Figure 3 and page 12, lines 6-7). In order to further clarify the scope of claim 24, this claim has been amended to recite "at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5." Thus, it is clear that amended claim 24 is directed to ARP3 polypeptides having at least 45% amino acid identity with the full-length human ARP3 sequence and does not encompass fragments of only two residues. In view of the above remarks and amendments, Applicant respectfully requests that the Examiner reconsider and remove this ground for rejecting the claims as allegedly lacking sufficient written description.

Regarding written description of structure rather than functional activity

The Examiner additionally emphasizes the *Lilly* decision, which held that a genus of nucleic acids defined merely by functional activity does not satisfy the written description requirement. Rather, a precise definition such as by structure, formula, chemical name or physical properties is required.

In regard to the Examiner's emphasis on *Lilly*, Applicant would respectfully point out that that claimed ARP3 polypeptides and fragments have not been defined by functional activity but, rather, have a precise structural definition. Specifically, each of the claimed ARP3 polypeptides and fragments has been described in reference to the structure of amino acid sequence SEQ ID NO:5, provided in Figure 3, rather than by a functional activity. The ARP3 polypeptide of claim 24 is structurally defined by containing an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5; the ARP3 polypeptide of claim 25 is structurally defined by containing the amino acid sequence of SEQ ID NO:5; and the ARP3 polypeptide fragment of claim 26 is structurally defined by containing at least ten contiguous amino acids of the amino acid sequence SEQ ID NO:5. Thus, the specification satisfies the written description requirement by disclosing a precise structural definition for each of the claimed ARP3 polypeptides and fragments.

In sum, Applicant submits that the specification satisfies the written description requirement and respectfully requests that the Examiner remove this rejection of claims 24 to 26 under the first paragraph of 35 U.S.C. § 112.

Regarding the Enablement Rejection under the first paragraph of 35 U.S.C. § 112

Applicant respectfully traverses the rejection of claims 24 to 26 under 35 U.S.C. § 112, first paragraph, and corresponding objection to the specification as allegedly lacking an enabling disclosure. The Office Action alleges that the claimed invention is not supported by a well established utility as required under 35 U.S.C. § 101 and concludes that one skilled in the art would not have known how to make and use the invention.

For the reasons presented above in regard to the § 101 rejection, Applicant submits that the claimed isolated ARP3 polypeptides and fragments have a specific, credible and substantial utility. In particular, the claimed ARP3 polypeptides and fragments can be useful, for example, for preparing antibodies to be used in imaging of metastatic prostate cancer. Based on the guidance provided in the specification and what is well known in the art, only routine work would have been required to make and use the claimed ARP3 polypeptides and fragments as antigens to prepare monoclonal antibodies or antisera. One skilled in the art would have been able to make the claimed polypeptides and fragments by routine

techniques and, further, would have been able to use the polypeptides and fragments to prepare antibodies using routine methodology based on the guidance provided in the specification. In this regard, the specification teaches that an ARP3 polypeptide useful as an immunogen can be prepared from natural sources or produced recombinantly (page 30, lines 1-25). Methods of conjugating an ARP polypeptide or fragment to a carrier molecule (page 30, lines 10-25) and guidance regarding well known techniques for preparing polyclonal or monoclonal antibodies are also provided in the specification (page 29, lines 8-12). As further guidance to the skilled person, the specification teaches that an anti-ARP3 binding agent such as a monoclonal antibody can be labeled, for example, with ^{111}indium, and imaging performed using radioimmunoscintography according to routine methods (specification at page 57, line 22, to page 58, line 2). In sum, in view of the guidance in the specification regarding how to make an ARP3 polypeptide or fragment and use the polypeptide or fragment to prepare an anti-ARP3 antibody or antiserum for use as an imaging agent for metastatic prostate cancer, only routine work, and not undue experimentation, would have been required to make and use the claimed polypeptides and fragments.

Regarding the scope of claims 24 and 26

The rejection of claims 24 and 26, and corresponding objection to the specification, as allegedly failing to enable the full scope of claims 24 and 26, is respectfully traversed. In making this rejection, the Office Action acknowledges that

the specification enables SEQ ID NO:5, yet asserts that "variants" of SEQ ID NO:5 are not enabled.

Claim 24 is drawn to an ARP3 polypeptide that contains an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5. As amended, claim 26 is directed to an ARP3 polypeptide fragment containing at least ten contiguous amino acids of SEQ ID NO:5. The Office Action asserts that claims 24 and 26 each include "variants" of SEQ ID NO:5 and that the specification does not show that variants of SEQ ID NO:5 are capable of functioning as SEQ ID NO:5.

Applicant respectfully disagrees with the assertions in the Office Action and submits that one skilled in the art would have been able to practice the full scope of the invention without undue experimentation. Specifically, based on the guidance provided in the specification and what was well known in the art, only routine work would have been required to make and use the claimed ARP3 polypeptides, for example, as antigens for preparation of monoclonal antibodies or antisera. As acknowledged in the Office Action, one skilled in the art would have been able to prepare a human ARP3 polypeptide having the amino acid sequence of SEQ ID NO:5, for example, by routine recombinant methods using the encoding nucleic acid sequence SEQ ID NO:4 (page 14, lines 11-20; page 30, lines 1-4). One skilled in the art similarly would have been able to prepare another ARP3 polypeptide encompassed by claim 24 using the guidance provided in the specification. In view of SEQ ID NO:4, which encodes human ARP3, one skilled in the art would have been able to isolate another species homolog such as a murine, bovine or

primate homolog using routine cloning methods (page 24, lines 27-30); as further taught in the specification, recombinant techniques can be used to prepare an ARP3 polypeptide having one or more amino acid substitutions, deletions or insertions as compared to SEQ ID NO:5 (page 24, line 31). As additional guidance to the skilled person, the specification teaches that an ARP3 polypeptide contains an amino acid sequence having at least 45% amino acid identity with SEQ ID NO:5 (page 24, lines 20-25) and can be conservatively or non-conservatively substituted as compared to the naturally occurring human ARP3 polypeptide sequence SEQ ID NO:5. Thus, in view of the guidance provided in the specification, only routine work such as standard recombinant techniques would have been required to isolate or prepare a variety of ARP3 polypeptides encompassed by claim 24.

Furthermore, the skilled person would have known that the use of an ARP3 polypeptide such as a non-human species homology would have been the same as the use of an ARP3 polypeptide having SEQ ID NO:5, and that well known techniques for preparing polyclonal or monoclonal antibodies can be used with any ARP3 polypeptide of the invention (page 29, lines 8-12). From the above, it is clear that undue experimentation would not have been required to make and use the ARP3 polypeptides of claim 24. Because the full scope of this claim is enabled by the specification, the Examiner is requested to remove this ground for rejecting claim 24 under 35 U.S.C. § 112, first paragraph.

One skilled in the art also would have been able to practice the full scope of claim 26 without undue experimentation. Claim 26 is directed to an ARP3 polypeptide fragment containing at least ten contiguous amino acids of SEQ ID NO:5. Thus, a polypeptide fragment of claim 26 contains an exact portion of the full-length ARP3 sequence, the portion having at least ten contiguous amino acids of the human ARP3 sequence SEQ ID NO:5. Thus, the subject matter of claim 26 does not encompass "variants" of SEQ ID NO:5 but rather is directed to sub-parts of the full-length native human sequence. All that would have been required for one skilled in the art to make and use the invention would have been to chemically synthesize a stretch of at least ten contiguous amino acids of SEQ ID NO:5, using the sequence of SEQ ID NO:5 provided in Figure 3, and to use such a fragment as an immunogen using routine techniques, for example, as set forth in the specification at page 29, lines 8-12, and page 30, lines 1-25. From the above, it is clear that only routine laboratory techniques, and not undue experimentation, would have been required to practice the full scope of claim 26.

In view of the above, Applicant submits that the claims are enabled by the specification and respectfully requests that the Examiner remove this ground for rejecting claims 24 to 26 under the first paragraph of 35 U.S.C. § 112.

Regarding the Rejections under 35 U.S.C. § 102

The §102(a) rejection over Rosen and Ruben

The rejection of claims 24 and 26 under 35 U.S.C. §102(a) as allegedly anticipated by Rosen and Ruben (AAB53386) is respectfully traversed. The Office Action indicates that the cited reference reports a sequence which is 100% similar to SEQ ID NO:5 from amino acid 372 to 537.

Regarding claim 24

Applicant submits that Rosen and Ruben do not teach the isolated ARP3 polypeptide of claim 24, which includes an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5. In particular, the human ARP3 sequence SEQ ID NO:5 is an amino acid sequence of 537 amino acids, as disclosed in the subject application in Figure 3. At best, Rosen and Ruben describe a protein sequence of 220 amino acids but do not teach an ARP3 polypeptide having the 537 amino acid sequence of SEQ ID NO:5, or an ARP3 polypeptide that includes an amino acid sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO: 5. Even if, for the sake of argument, Rosen and Ruben reported a sequence having 100% amino acid identity with residues 372 to 537 of SEQ ID NO:5, such a sequence has only about 30% amino acid identity with the 537 amino acid sequence SEQ ID NO: 5 and, therefore, cannot anticipate the invention.

While Applicant maintains that the claims are novel over the cited art, claim 24 has been amended to indicate that the claimed polypeptide includes an amino acid sequence having at least 45% amino acid identity "with residues 1 to 537 of SEQ ID NO:5." The amendment to claim 24 is made solely in order to expedite prosecution of the subject application and does not change the scope of claim 24.

Regarding Claim 26

As indicated above, claim 26 is directed to an ARP3 polypeptide fragment containing at least ten contiguous amino acids of SEQ ID NO:5. Applicant submits that the AAB53386 sequence by Rosen and Ruben is not prior art with respect to the claimed invention because Applicant reduced the invention to practice prior to September 21, 2000, when AAB53386 was published. As evidence that publication of AAB53386 by Rosen and Ruben was not before Applicant's date of invention, Applicant submits herewith a Declaration under 37 C.F. R. § 1.131 along with copies of relevant documentary evidence. The dates on the documentary evidence have been redacted; however, the dates shown in the original documents indicate that Applicant had obtained a nucleic acid sequence encoding an ARP3 polypeptide fragment including residues 372 to 537 of SEQ ID NO:5 prior to September 21, 2000.

Because the Rosen and Ruben AAB53386 sequence was not published more than one year prior to the priority date of the above-identified application and because Applicant reduced the invention to practice before the cited reference was published, this reference is not prior art with respect to the claimed

invention. In view of the above remarks, Applicant respectfully requests that the Examiner remove the rejection of claims 24 and 26 under 35 U.S.C. §102(a) as allegedly lacking novelty over Rosen and Ruben.

The §102(b) rejection over Emerson et al.

The rejection of claims 24 and 26 under 35 U.S.C. §102(b) as allegedly anticipated by Emerson et al. (AAW93405) is respectfully traversed. The Office Action asserts that Emerson et al. report a sequence which is 100% similar to SEQ ID NO:5 from amino acids 327-334.

Regarding claim 24

For the reasons set forth above in regard to Rosen and Ruben, the cited art by Emerson et al. does not describe a sequence having at least 45% amino acid identity with residues 1 to 537 of SEQ ID NO:5. Thus, Emerson et al. cannot anticipate the ARP3 polypeptide of claim 24.

Regarding claim 26

The cited reference by Emerson et al. at best describes a short sequence of 8 residues. However, this sequence cannot anticipate the ARP3 polypeptide fragment of claim 26, which as amended includes at least ten contiguous amino acids of SEQ ID NO:5. Consequently, claim 26 is novel over Emerson et al. Applicant therefore respectfully requests that the Examiner remove the rejection of claims 24 and 26 under 35 U.S.C. §102(b) as allegedly anticipated by Emerson et al.

CONCLUSION

In view of the above remarks, Applicant respectfully requests that the Examiner reconsider and allow the pending claims. The Examiner is invited to call the undersigned agent or Cathryn Campbell if there are any questions.

Respectfully submitted,

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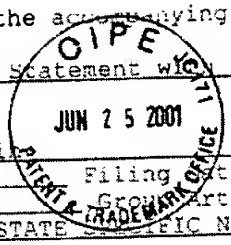
Please acknowledge receipt of the accompanying:

☒ Other Information Disclosure Statement w/Ex. PTO Form 1449 and 39 references



Applicant's Name Biaoyang Lin
Serial Number 09/821,812 Filing Date 03/28/2001
Examiner's Name Unknown Group Art Unit 1646
Title ANDROGEN REGULATED PROSTATE SPECIFIC NUCLEIC ACIDS

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EXHIBIT A

to induce histone deacetylase-mediated gene silencing[21,22]. This suggests an attractive model for temporal specification in the *Drosophila* CNS, in which Hb recruits Mi-2-Polycomb to silence genes conferring later-born temporal identity. The molecular basis of competence remains unclear, with one exception: we know that downregulation of Hb triggers progressive loss of competence, because maintaining continuous high Hb levels can indefinitely maintain competence[11]. Is competence due to the presence of an unknown Hb target and cofactor, or the absence of a negative factor that suppresses early-born fates (such as Klumpfuss[23])? Does loss of competence in old NBs and in differentiating neurons occur by different mechanisms? Is there a distinct, later competence state for specifying subsequent Krüppel-positive temporal identities? *Drosophila* NBs now provide a model system for investigating the molecular nature of neural progenitor competence. □

Methods

We used the following fly stocks: (1) *hsp70-hb* (HB476.1 homozygous on chromosome III); (2) *yw; UAS-hb; UAS-hb*; (3) *yw; +; UAS-hb*; (4) *prospero-Gal4/prospero-Gal4* on chromosome III; (5) *prospero-Gal4/CyO, ftz-lacZ; eve-tau::lacZ/TM3, ftz-lacZ*; (6) *eve[+3.5–4.3]-Gal4/eve[+3.5–4.3]-Gal4* on chromosome II; (7) *engrailed-Gal4/engrailed-Gal4* on chromosome II; (8) *yw, hsp70-FLP* on chromosome X; and (9) *+/Act5c-FRT-stop-FRT-Tau::lacZ, CyO*.

For *hs-hb* experiments, embryos were collected for 1–2 h, aged to the indicated time, and subjected to three cycles of 30 min at 37 °C then 1 h at 22 °C, and allowed to develop to stage 16–17. This generates the maximum Hb level without affecting control embryos: Hb protein is detected throughout the CNS for 4 h after induction. Before heat shock, some embryos were fixed and stained with Eve/Hb for developmental staging. For *hs-FLP* experiments, embryos were subjected to heat shock for 22 min at 37 °C and aged to stage 16–17. Detailed methods available upon request.

All antibodies, staining procedures and imaging methods have been described previously[11], except for guinea-pig anti-Runt (1:500) and rat anti-Vvl (1:100) antibodies. All images were collected as confocal image stacks, processed in ImageJ (NIH), and shown as two-dimensional projections. U neurons are shown as insets in their approximate spatial position if they would be obscured in the projection.

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Basal body dysfunction is a likely cause of pleiotropic Bardet–Biedl syndrome

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Bardet–Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized primarily by retinal dystrophy, obesity, polydactyly, renal malformations and learning disabilities. Although five BBS genes have been cloned[1–4], the molecular basis of this syndrome remains elusive. Here we show that BBS is probably caused by a defect at the basal body of ciliated cells. We have cloned a new BBS gene, *BBS8*, which encodes a protein with a prokaryotic domain, *pilF*, involved in pilus formation and twitching mobility. In one family, a homozygous null *BBS8* mutation leads to BBS with randomization of left–right body axis symmetry, a known defect of the nodal cilium. We have also found that BBS8 localizes specifically to ciliated structures, such as the connecting cilium of the retina and columnar epithelial cells in the lung. In cells, BBS8 localizes to centrosomes and basal bodies and interacts with PCM1, a protein probably involved in ciliogenesis. Finally, we demonstrate that all available *Caenorhabditis elegans BBS* homologues are expressed exclusively in ciliated neurons, and contain regulatory elements for RFX, a transcription factor that modulates the expression of genes associated with ciliogenesis and intraflagellar transport.

BBS exhibits substantial genetic heterogeneity and, although typically inherited in an autosomal recessive pattern, in some

EXHIBIT B

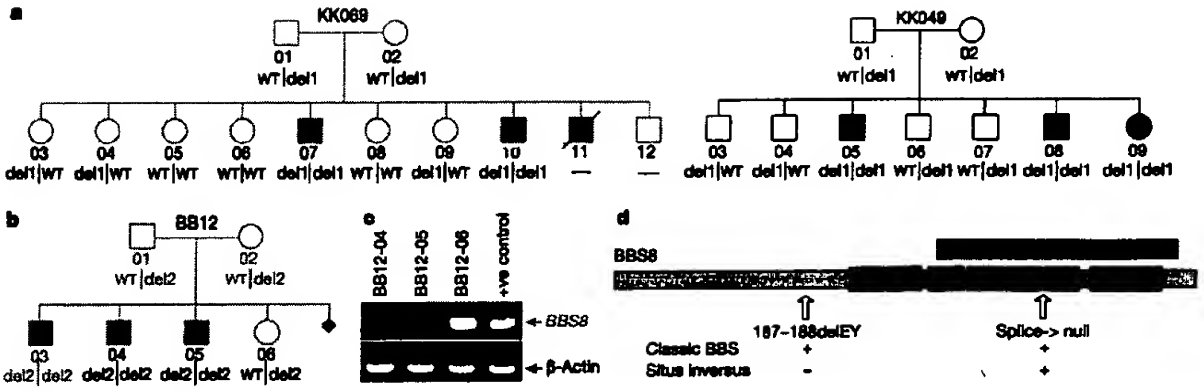

Figure 1 *BBS8* causes BBS. **a**, In families KK049 and KK069, affected individuals are homozygous for a 6-bp deletion that eliminates residues 187–188. Squares, males; circles, females. **b**, In pedigree BB12 all patients are homozygous for a 3-bp deletion that abolishes the splice donor site in intron 10. **c**, RT–PCR from tubular kidney cells cultured from patients BB12-04, -05 and -06. *BBS8* messenger RNA is detected in the unaffected sister and the unrelated control but not the patients. **d**, Predicted domain structure of BBS8 with TPRs depicted as boxes. The C terminus of BBS8 is predicted to contain a *pilF* domain (blue box). The positions of the mutations are shown (arrows).

cases three mutant alleles in two genes are required for pathogenesis[7–10]. Recently, a phylogenetic/genomic approach that identifies distant paralogues of known BBS proteins was successful in cloning new BBS loci[1]. For the present study, we fragmented BBS4 into eight overlapping segments and searched the conceptual translation of the draft human genome and dbEST. Because BBS4 contains at least ten tetratricopeptide repeats (TPRs), we identified numerous TPR-containing transcripts. In one instance, however, we observed an alignment between three consecutive TPRs of BBS4 and a contiguous region of the hypothetical protein TTC8 (Supplementary Fig. 1a).

TTC8 maps to chromosome 14q32.11 (Supplementary Fig. 1b), a region that does not contain a mapped BBS locus. Nevertheless, on the basis of its similarity to BBS4, we hypothesized that TTC8 might be involved in BBS. We tested this by screening *TTC8* in a cohort of 120 unrelated BBS patients. We identified homozygous alterations in patients from three families: further sequencing and clinical evaluations showed that all eight individuals affected in these families have a homozygous mutant genotype and exhibit classical BBS signs (Fig. 1a, b; see also Supplementary Fig. 2a and Table 1). In families KK049 and KK069 of Saudi Arabian lineage, each affected individual, but no unaffected relative, was homozygous for a 6-base-pair (bp) in-frame deletion in exon 6 that eliminates two amino acids (187–188delEY) (Fig. 1a; see also Supplementary Fig. 2a). We also detected a statistically significant association between the phenotype in these two families and *TTC8* at $\theta = 0$; two-point linkage analyses gave a LOD score from 3.36 (90% penetrance) to 3.54 (99% penetrance). Although the 187–188delEY allele might be in linkage disequilibrium with the true pathogenic mutation, we found either an E residue or a conservative substitution at position 187 in all 16 identifiable TTC8 homologues—we observed similar conservation for the Y residue (14 of 16; 87.5%). More importantly, in all 16 species with the TTC8 homologue, at least one of the two amino acids is conserved (Supplementary Fig. 2b). In a third consanguineous family of Pakistani descent (BB12) we identified a homozygous 3-bp deletion that abolishes the donor sequence at the splice junction of exon 10 (IVS10 + 2–4delTGC) in all three patients but not their unaffected sister (Fig. 1b). The expected effect of this mutation is a read-through culminating in an intronic stop codon. To substantiate this prediction, we cultured renal tubular cells and

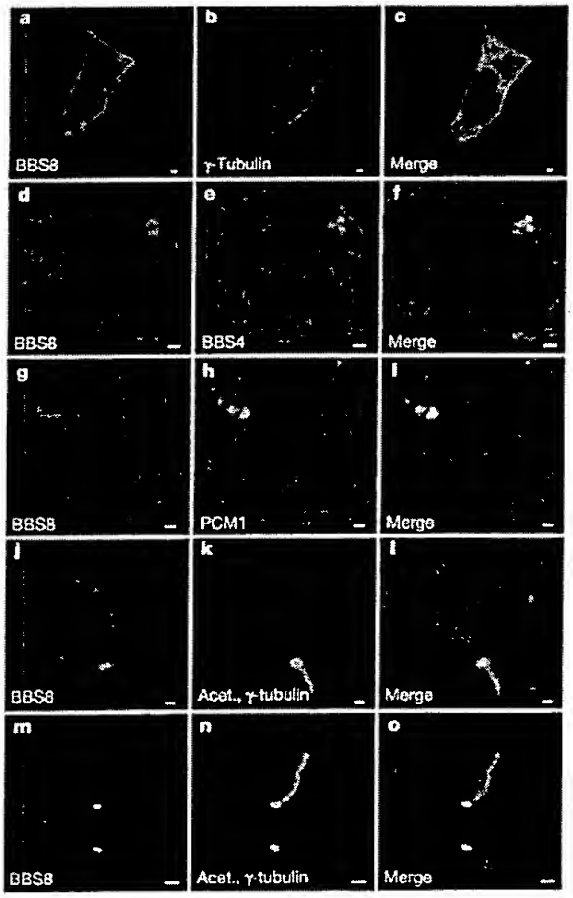

Figure 2 BBS8 is a centrosomal and basal body protein. **a–l**, BBS8 co-localizes with γ-tubulin (**a–c**; HEK293), BBS4 (**d–f**; HeLa) and PCM1 (**g–i**; HeLa). **j–o**, In ciliated cells BBS8 localizes to the basal body in NIH3T3 cells (**j–l**) and to both basal bodies and centrosomes in IMCD3 cells (**m–o**). Scale bar, 1 μm. Acet., acetylated.

skin fibroblasts from patients BB12-04 and -05 and their unaffected sister BB12-06—who is heterozygous for the mutation—and performed polymerase chain reaction with reverse transcription (RT–PCR). We detected *BBS8* expression in BB12-06 and an unrelated control cell line, but not in the two BBS patients (Fig. 1c), suggesting that the aberrant *BBS8* transcript is probably eliminated by nonsense-mediated decay. These data, together with the absence of these alterations from 192 ethnically matched control chromosomes, led us to conclude that mutations in *TTC8* cause BBS, thus defining the eighth locus for this disorder, *BBS8*.

BBS8 generates two alternatively spliced isoforms (Supplementary Fig. 1b) and is widely expressed (Supplementary Fig. 3). The predicted protein contains eight TPRs towards the carboxy terminus and exhibits significant ($P < 10^{-5}$) similarity to a prokaryotic domain *pilF* (Fig. 1d; see also Supplementary Fig. 4), involved in twitching mobility and type-IV pilus assembly[11,12], raising the possibility that BBS8 might be relevant to the function of cilia, flagella or pseudopodia. Notably, one of the patients in family BB12 manifests situs inversus, a known defect of left–right axis determination caused by dysfunction of the nodal cilium[13]. The association between BBS and the situs inversus phenotype is not coincidental (see Supplementary Fig. 5 and legend), although the presence of situs in only one of the three BB12 patients suggests that the defect is one of randomization of left–right symmetry.

To investigate BBS8 further, we determined the localization of the protein in cells and tissues. Immunocytochemical analyses of HeLa and HEK293 cells identified BBS8 as a centrosomal protein with a localization signal overlapping with γ-tubulin, an established centrosomal marker (Fig. 2a–c). This was reminiscent of the intracellular pattern of BBS4 (Kim, J. C. *et al.*, manuscript submitted); double staining with antibodies against BBS8 and BBS4 showed co-localization near the centrosome (Fig. 2d–f). In ciliated NIH3T3 cells we found the protein at the base of the cilium (Fig. 2j–l), whereas in kidney IMCD3 cells, we found BBS8 tightly

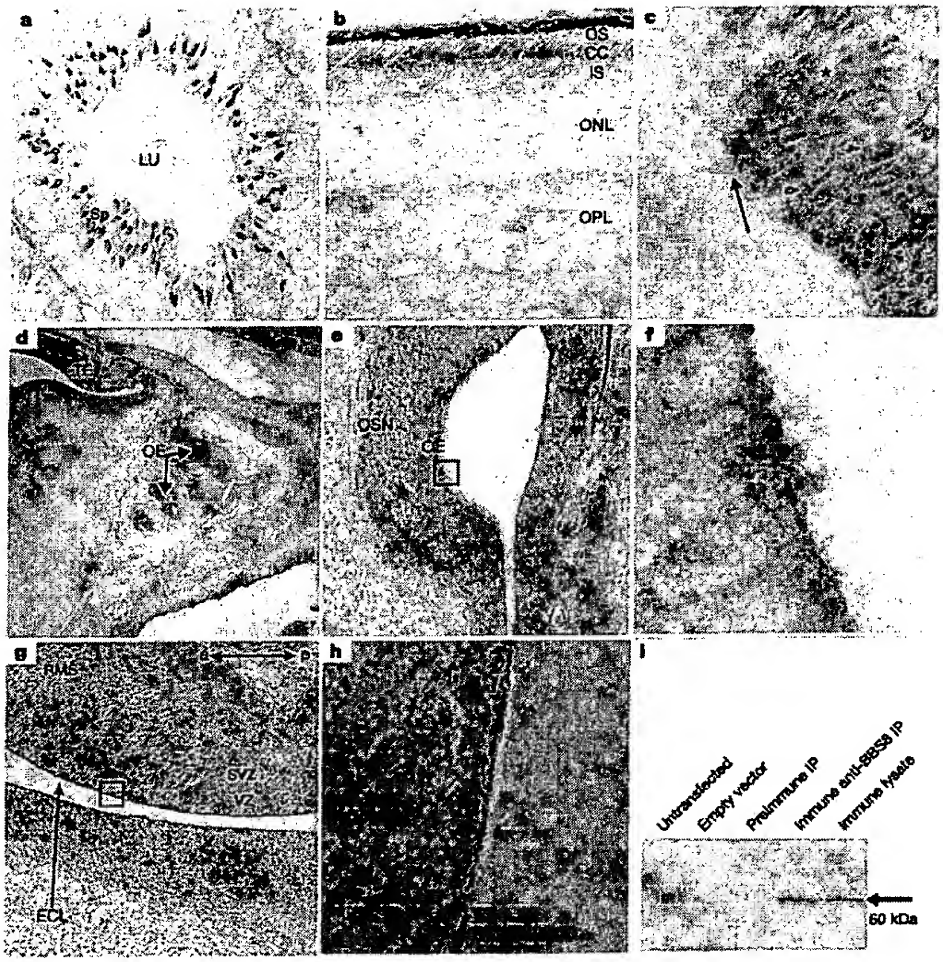

Figure 3 BBS8 localizes to ciliated structures in tissues. **a**, Maturing (stages X and XI), flagellated spermatids (Sp) surrounding the lumen (LU) of a 12 day (P12) mouse seminiferous tubule. **b**, Connecting cilium (CC) of a P12 mouse retina; no protein is detectable in outer or inner segments (OS, IS), outer nuclear layer (ONL), or outer plexiform layer (OPL). **c**, Adult human bronchial epithelium with prominent expression in ciliated columnar epithelial cells but not goblet cells (asterisk); the ciliary border is indicated (arrow). **d**, Sagittal section of an E14 mouse embryo showing BBS8 staining in the olfactory epithelium (OE) and telencephalon (TE). **e**, Sagittal section of an E16 sinus, with BBS8 localizing to the olfactory epithelium (OE) and olfactory sensory neurons (OSN). **f**, Higher magnification of boxed area in **e**. **g**, BBS8 localizes to the ventricular and subventricular zones (VZ, SVZ) and the rostral migratory stream (RMS) of the anterior telencephalon (sagittal section, E16). Strong expression is visible in the ependymal cell layer (ECL, arrow). **h**, Higher magnification of box from **g**. **i**, Western blot showing specificity of the anti-BBS8 antibody. IP, immunoprecipitate.

associated with both the basal body and the centrosome, but not the ciliary axoneme (Fig. 2m–o).

We also queried whether BBS8 interacts with the other known BBS proteins or their interactors. Of particular interest was the BBS4-interacting protein PCM1 (Kim, J. C. *et al.*, manuscript submitted), a centrosomal protein also found in ciliary basal bodies[14,15] and probably involved in centriolar replication during ciliogenesis[15]. Immunoprecipitations indicated that BBS8 binds to the C terminus of PCM1 (Supplementary Fig. 6). BBS8 also co-localizes with PCM1, confirming the immunoprecipitation data (Fig. 2g–i).

We next investigated the distribution of BBS8 in tissues. We raised a polyclonal antibody to human BBS8, which, based on its peptide sequence, is also expected to cross-react with its mouse orthologue. We detected specific staining of ciliated structures in 12-day-old mice (P12), including maturing (stages X and XI) spermatids (Fig. 3a), the connecting cilium of the retina (Fig. 3b) and bronchial epithelial cells (Fig. 3c). In mouse embryos at 14 and 16 days old (E14 and E16), we also detected specific localization in the telencephalon, with prominent staining at the developing ependymal cell layer (which is ciliated) and the olfactory epithelium (Fig. 3d–h). Immunoprecipitation with the anti-BBS8 antiserum of protein extracts of Myc–BBS8-transfected HEK293 cells followed by western blotting revealed that the anti-BBS8 immune serum, but not the preimmune serum, immunoprecipitated specifically Myc–BBS8 (Fig. 3i).

To investigate further our hypothesis that BBS8 is associated with ciliary biogenesis or function, we used the nematode *C. elegans*, an important eukaryotic model system for ciliation and intraflagellar transport (IFT) studies[16]. Of the 959 somatic cells that make up an adult hermaphrodite, 302 are neuronal and only a subset (60) have ciliated dendritic endings.

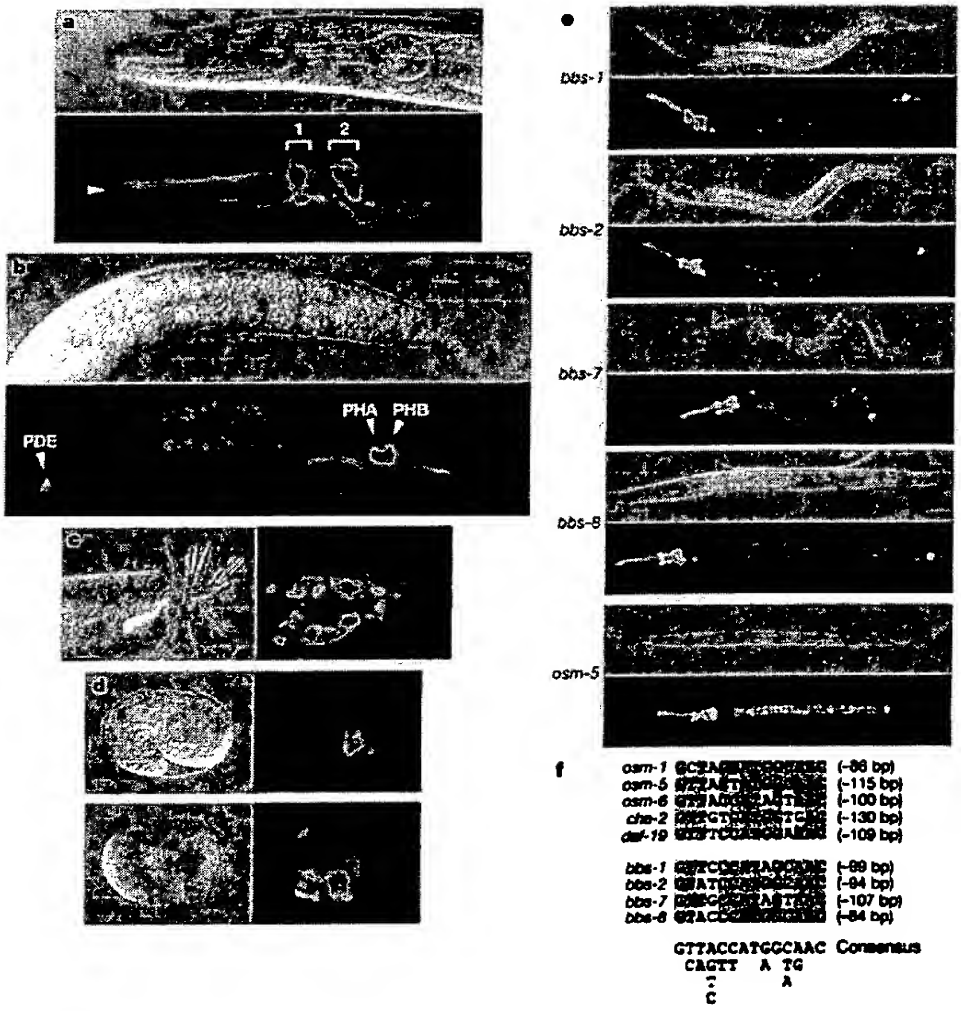

Figure 4 *Caenorhabditis elegans bbs* genes are expressed exclusively in ciliated neurons. **a–d**, DIC and GFP images of a nematode bearing a transcriptional *C. elegans bbs8*::GFP reporter (DIC image shown first). **a**, Head region of an L1/L2 animal showing strong expression in inner/outer labial neurons (1) and in amphid head neurons (2). Dendritic processes extend anteriorly to the nose (arrowhead). **b**, Tail region of an L3/L4 animal showing expression in PHA and PHB tail neurons and PDE neuronal cell. **c**, Adult male showing expression in tail sensory ray neurons. **d**, *bbs-8* expression at the 1.5-fold (upper panel) and 3.0-fold (lower panel) embryonic stages. **e**, Transcriptional GFP reporter constructs of all *bbs* genes and *osm-5* in L1/L2 animals. **f**, Alignment of X boxes from *C. elegans* genes expressed in ciliated neurons and all *bbs* genes. Distances after the X boxes are from the translation start site (ATG) of each gene.

BLAST analysis of the *C. elegans* genome and proteome with BBS8 detected significant ($P < 10^{-8}$) similarity to a single predicted transcript, T25F10.5. To ascertain whether this gene is expressed in ciliated cells, we generated a transcriptional green fluorescent protein (GFP) expression construct and analysed transgenic lines carrying extrachromosomal arrays for GFP fluorescence. The expression pattern observed was indistinguishable from that of genes (for example, *osm-5*, the *C. elegans* orthologue of the mouse *Tg737* polycystic kidney disease gene[17]) whose spatial distribution is restricted to ciliated cells (Fig. 4a, b, e). Expression in hermaphrodites was most prominent in the head region (amphid and inner/outer labial neurons), in the tail region (in both phasmid neurons), and in the mid-body ciliated neuronal cell PDE (Fig. 4a, b). We also detected strong GFP signals in the male tail-ray neurons, all of which are ciliated (Fig. 4c). GFP expression was not detected in non-ciliated interneurons or motor neurons of the hermaphrodite tail or mid-body, or in any other tissues. The earliest point during development where expression was observed, at the 1.5-fold stage (Fig. 4d, upper panel), correlates with the onset of ciliogenesis[18], and expression levels predictably increase as embryos develop to the threefold stage (Fig. 4d, lower panel).

Given that mutations in all known BBS genes give rise to identical or overlapping phenotypes, we postulated that all BBS proteins might be involved in the same cellular function. If this is true and the expression of the *C. elegans bbs-8* is relevant to the cellular mechanism of this disorder, other *BBS* orthologues should exhibit the same specific expression pattern. We investigated this by identifying the *C. elegans* orthologues of *BBS1*, *BBS2* and *BBS7* and determining their expression pattern. Consistent with our hypothesis, transgenic lines containing the three transcriptional *bbs*::GFP constructs showed expression patterns restricted exclusively to ciliated neurons and identical to that of *bbs-8* and *osm-5* (Fig. 4e).

Our data also suggest that each *C. elegans* BBS orthologue is regulated by the same mechanism. Computational analyses of the 5′ untranslated regions (UTRs) of *bbs-1*, *bbs-2*, *bbs-7* and *bbs-8* identified a common regulatory element also present in numerous genes expressed in ciliated neurons (Fig. 4f). This motif, a 14-bp imperfect repeat termed X box, is centred typically about 100 bp upstream of the start codon[19]. The transcription factor regulating the expression of X boxes in *C. elegans*, DAF-19, is a member of the RFX protein family and is required for cilia formation[19].

Collectively our data indicate a role for the BBS proteins in ciliary function. The BBS phenotype is consistent with this hypothesis, as many clinical aspects of this disorder can be explained by a ciliary defect. Dysfunction of the nodal cilium causes left–right axis defects in vertebrates[13,20,21]. Also, compromised protein transport across the photoreceptor-connecting cilium causes retinal dystrophy[21,22], whereas failure of mechanosensation at the primary cilium of renal tubular cells causes polycystic kidney disease (PKD)[23]. The best-studied mouse models of PKD, the Oak Ridge polycystic kidney mutant (*Tg737*^{orpk}) and *cpk*, both develop BBS-like renal phenotypes, including renal cysts, with pancreatic and hepatic involvement[21,24].

Despite the phenotypic similarities with other ciliary defects, BBS is probably caused by a potentially new mechanism. BBS8 is not found in the axoneme and is therefore unlikely to be an IFT or structural axonemal protein. Rather, the specific localization of BBS8 to the basal body and its interaction with PCM1 raise the possibility that it either participates in ciliogenesis or mediates communication between the cilium and the interior of the cell. Intriguingly, BBS8 bears similarity to yeast *cdc23* (data not shown), a member of the anaphase-promoting complex. Inversin, another protein involved in ciliation and left–right axis determination, binds to the mammalian anaphase-promoting complex and has been postulated to be involved in the cell cycle[25,26]. Our findings may thus facilitate the understanding of the link between ciliary function and cellular response and provide an entry point to understanding the developmental and cognitive defects of BBS. □

Methods

Bardet–Biedl syndrome patients

R.A.L. and P.L.B. collated clinical data, performed clinical examinations and secured a diagnosis of BBS according to established criteria[27]. Blood and clinical data were obtained with consent, in accordance with protocols approved by the human subjects ethics committees at each participating institution, and DNA was purified as described[1].

Mutation analyses

We determined the *BBS8* intron–exon structure by aligning its predicted complementary DNA sequence to the draft human genome as described[1]. PCR products from BBS patients, relatives and ethnically matched controls were sequenced[1]; primer sequences are available on request.

Expression studies

We performed RT–PCR and northern analyses with a 443-bp amplicon from the 3′ UTR or a full-length cDNA clone of *BBS8* as described[1]. Proximal tubular cells were selectively grown from urine samples in modified DMEM F12/10% FCS medium (Invitrogen) as were fibroblasts in HAM F10/12% FCS medium (GibcoBRL). After monolayer confluence, cells were trypsinized and RNA was prepared with Trizol (GibcoBRL) (1 ml cm^{-2}). First-strand cDNA was synthesized by random priming with reverse transcriptase (Invitrogen). Complementary DNA was amplified with internal exonic primers corresponding to exons 9 and 13.

Immunocytochemistry

The open reading frame of human *BBS8* was cloned into the pCMV–Myc vector (BD Biosciences) and confirmed by double-strand sequencing. We cultured HeLa or HEK293 cells on 18-mm coverslips to 70–80% confluency and transfected them with a Myc–BBS8 plasmid or a GFP–BBS8 plasmid using the calcium phosphate kit (Invitrogen) or Polyfect transfection reagent (Qiagen). Cells were collected 24 h after transfection, fixed in −20 °C methanol, permeabilized in 0.1% Triton X-100, and blocked with phosphate buffer saline (PBS) containing 5.5% fetal bovine serum. We incubated the cells with anti-Myc mouse monoclonal antibody (Clontech). After washing with PBS, we incubated cells with a secondary rabbit anti-mouse antibody conjugated with Alexa Fluor 488 or 594 fluorophore (Molecular Probes). We used 4,6-diamidino-2-phenylindole to stain DNA, and coverslips were mounted in prolong antifade reagent (Molecular Probes). For triple-staining experiments, we used a γ-tubulin monoclonal antibody (Molecular Probes) or a PCM1 rabbit polyclonal antibody (a gift from A. Merdes) and a goat anti-rabbit secondary antibody conjugated with Alexa Fluor 488 or 594 dyes. Cilia were visualized with an acetylated tubulin monoclonal antibody (Sigma).

Antibodies and immunohistochemistry

We generated a polyclonal BBS8 antibody by injecting rabbits with the amino terminal (position 111–126) peptide GFLRPSTQSGRPGTME. This antibody was used in immunohistochemistry of 5-μm-thick sagittal sections from mouse embryos and adult mouse and human tissues (Novagen) according to standard protocols. The primary anti-BBS8 antibody was used in 1:1,000 and 1:2,000 dilutions in block, applied to the slides and incubated overnight at 4 °C. Staining was detected with the ABC complex (DAKO) and DAB (Biomeda) according to the manufacturer's instructions.

Immunoprecipitations

HEK293 cells grown in 100-mm tissue culture dishes were transfected transiently with Myc–BBS8 and haemagglutinin–PCM1 (encoding the C terminus of the protein from amino acid 1574 to the end) with the calcium phosphate kit (Invitrogen). Cells were collected 48 h after transfection in co-immunoprecipitation buffer (150 mM NaCl; 50 mM Tris-HCl pH 7.5; 1% NP-40) supplemented with protease inhibitor (Roche) and 500 μl of 100 mM sodium orthovanadate. Cell lysates were incubated overnight with 10 μg anti-Myc monoclonal antibody (immunoprecipitate) immobilized onto Sepharose beads (Covance). The immunoprecipitates were washed three times with co-immunoprecipitation buffer, resuspended in loading buffer and used in western blots. Immunoblot PVDF membranes (Bio-Rad) were blocked with 0.2% Tween 20 in PBS and 5% milk, probed with an anti-haemagglutinin mouse monoclonal antibody coupled to peroxidase (Roche), and detection was performed using an ECL system (Amersham-Pharmacia).

Caenorhabditis elegans bbs::GFP expression constructs

Transcriptional GFP expression constructs, generated as PCR products using a PCR-fusion-based approach[28], were constructed by placing the upstream UTR of *C. elegans* genes Y105E8A.5 (*bbs-1*), F20D12.3 (*bbs-2*), Y75B8A.12 (*bbs-7*) and T25F10.5 (*bbs-8*) 5′ to a DNA fragment containing GFP and the UNC-54 3′ UTR. Specifically, the *bbs* constructs contain 500 bp of upstream sequence and the first 15 bp of exon 1 for *bbs-1*; 251 bp UTR and first 14 bp of exon 1 for *bbs-2*; 1,559 bp UTR and first 15 bp of exon 1 for *bbs-7*; and 370 bp UTR and first 16 bp of exon 1 for *bbs-8*. The GFP–UNC-54 3′ UTR fragment, which also contains a nuclear localization signal sequence 5′ to GFP, was amplified from the GFP expression vector, pPD95.67, provided by A. Fire. Transgenic lines carrying extrachromosomal arrays of the *bbs*::GFP expression constructs were generated by coinjection of *dpy-5(e907)* nematodes with the GFP construct and a rescue plasmid, pCeh 341, which contains wild-type *dpy-5* (provided by C. Thacker and A. Rose). The *osm-5*::GFP construct was obtained from B. K. Yoder. All nematodes were cultured as described previously[29].

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Authors' contributions The laboratories of M.R.L., P.L.B. and N.K. contributed equally to this work.

Competing interests statement The authors declare that they have no competing financial interests.

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Nucleotide sequences for the two *BBS8* splice isoforms (AY366523 (long isoform) and AY366524 (short isoform)) have been deposited in GenBank.

The Wnt/β-catenin pathway regulates cardiac valve formation

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Truncation of the tumour suppressor adenomatous polyposis coli (Apc) constitutively activates the Wnt/β-catenin signalling pathway[1]. Apc has a role in development: for example, embryos of mice with truncated Apc do not complete gastrulation[2]. To understand this role more fully, we examined the effect of truncated Apc on zebrafish development. Here we show that, in contrast to mice, zebrafish do complete gastrulation. However, mutant hearts fail to loop and form excessive endocardial cushions. Conversely, overexpression of Apc or Dickkopf 1 (Dkk1), a secreted Wnt inhibitor[3], blocks cushion formation. In wild-type hearts, nuclear β-catenin, the hallmark of activated canonical Wnt signalling[4], accumulates only in valve-forming cells, where it can activate a Tcf reporter. In mutant hearts, all cells display nuclear β-catenin and Tcf reporter activity, while valve markers are markedly upregulated. Concomitantly, proliferation and epithelial–mesenchymal transition, normally restricted to endocardial cushions, occur throughout the endocardium. Our findings identify a novel role for Wnt/β-catenin signalling in determining endocardial cell fate.

Apc is an essential component of the axin-containing destruction complex that phosphorylates β-catenin, tagging it for ubiquitination and degradation by the proteasome. In the presence of a Wnt ligand, β-catenin is stabilized and accumulates in the nucleus where it binds and activates Tcf transcription factors[1]. APC mutations, common in colorectal cancer, occur proximal to the axin-binding motifs in the mutation cluster region (MCR; Fig. 1a). These truncations lead to constitutive activation of the pathway.

We have recently developed a reverse genetics strategy for inactivating genes in the zebrafish germline[5]. The current zebrafish genome database contains a single *apc* orthologue (Supplementary Fig. 1a, b). We screened an F_1 *N*-ethyl-*N*-nitrosourea (ENU)-mutagenized zebrafish library for apc nonsense mutations mapping to the putative MCR. A premature stop codon corresponding to amino acid (a.a.) 1318 of human APC was identified. The allele was designated apc^{mcr}, and is predicted to constitutively activate Wnt/β-catenin signalling.

apc^{mcr} heterozygotes developed normally. Intercrossing resulted in clutches of F_3 embryos of which 25% died between 72 and 96 hours post-fertilization (h.p.f.), displaying multiple defects. These included, most prominently, cardiac malformation with associated pericardial oedema and blood pooling (Fig. 1b), enlarged otic vesicles, smaller eyes, and body curvature. Further, jaw, pharynx, and inner-ear structures failed to develop and fin buds arrested. Primordia for internal organs such as gut, liver and pancreas formed but developed abnormally (A.F.L.H and A-P.G.H, unpublished observations). Genotyping revealed complete correspondence between this phenotype and homozygosity for the apc^{mcr} mutation. Mutant embryos probably developed beyond gastrulation owing to the presence of maternal Apc (data not shown).

To verify that the above developmental defects were due to loss of Apc function and not to co-segregation of an unidentified linked mutation, we injected zygotes resulting from intercrosses

Alignment of Seq ID N0. 5 and AY366523 Protein

Sequence Range: 1 to 537

```
                  10        20        30        40        50
Seq.No.5    GGLTTTVIGTRLGVDRPRLSWSAGPSLAAPAAMSSEMEPLLLAWSYFRRR
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

                                                  10
AY366523_p                                    MSSEMEPLLLAWSYFRRR>
                                              ||||||||||||||||||
Seq.No.5                                      MSSEMEPLLLAWSYFRRR

              460       470       480       490       500
AY366523_p    HFNFATISDKIGDLQRSYVAAQKSEAAFPDHVDTQHLIKQLRQHFAML>
                    |    | |   ||  | || |      | |   | |
Seq.No.5      LTTTVIGTRLGVDRPRLSWSAGPSLAAPAAMSSEMEPLLLAWSYFRRR


                  60        70        80        90        100
Seq.No.5    KFQLCADLCTQMLEKSPYDQAAWILKARALTEMVYIDEIDVDQEGIAEMM
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

            20        30        40        50        60
AY366523_p  KFQLCADLCTQMLEKSPYDQAAWILKARALTEMVYIDEIDVDQEGIAEMM>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    KFQLCADLCTQMLEKSPYDQAAWILKARALTEMVYIDEIDVDQEGIAEMM


                 110       120       130       140       150
Seq.No.5    LDENAIAQVPRPGTSLKLPGTNQTGGPSQAVRPITQAGRPITGFLRPSTQ
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

            70        80        90        100       110
AY366523_p  LDENAIAQVPRPGTSLKLPGTNQTGGPSQAVRPITQAGRPITGFLRPSTQ>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    LDENAIAQVPRPGTSLKLPGTNQTGGPSQAVRPITQAGRPITGFLRPSTQ


                 160       170       180       190       200
Seq.No.5    SGRPGTMEQAIRTPRTAYTARPITSSSGRFVRLGTASMLTSPDGPFINLS
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

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AY366523_p  SGRPGTMEQAIRTPRTAYTARPITSSSGRFVRLGTASMLTSPDGPFINLS>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    SGRPGTMEQAIRTPRTAYTARPITSSSGRFVRLGTASMLTSPDGPFINLS


                 210       220       230       240       250
Seq.No.5    RLNLTKYSQKPKLAKACLSISFIMKMMLRLLWIWLALSTEHSQYKDWWWK
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

          170       180
AY366523_p  RLNLTKYSQKPKLAKAL>
            |||||||||||||||||
Seq.No.5    RLNLTKYSQKPKLAKAC


                                     200       210
AY366523_p                         ENDVKTALDLAALSTEHSQYKDWWWK>
                                     ||      ||||||||||||||||
Seq.No.5                           KMMLRLLWIWLALSTEHSQYKDWWWK


                 260       270       280       290       300
Seq.No.5    VQIGKCYYRLGMYREAEKQFKSALKQQEMVDTFLYLAKVYVSLDQPVTAL
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>
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EXHIBIT C

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          220       230       240       250       260
AY366523_p  VQIGKCYYRLGMYREAEKQFKSALKQQEMVDTFLYLAKVYVSLDQPVTAL>
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    VQIGKCYYRLGMYREAEKQFKSALKQQEMVDTFLYLAKVYVSLDQPVTAL

                  310       320       330       340       350
Seq.No.5    NLFKQGLDKFPGEVTLLCGIARIYEEMNNMSSAAEYYKEVLKQDNTHVEA
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

          270       280       290       300       310
AY366523_p  NLFKQGLDKFPGEVTLLCGIARIYEEMNNMSSAAEYYKEVLKQDNTHVEA>
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    NLFKQGLDKFPGEVTLLCGIARIYEEMNNMSSAAEYYKEVLKQDNTHVEA

                  360       370       380       390       400
Seq.No.5    IACIGSNHFYSDQPEIALRFYRRLLQMGIYNGQLFNNLGLCCFYAQQYDM
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

          320       330       340       350       360
AY366523_p  IACIGSNHFYSDQPEIALRFYRRLLQMGIYNGQLFNNLGLCCFYAQQYDM>
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    IACIGSNHFYSDQPEIALRFYRRLLQMGIYNGQLFNNLGLCCFYAQQYDM

                  410       420       430       440       450
Seq.No.5    TLTSFERALSLAENEEEAADVWYNLGHVAVGIGDTNLAHQCFRLALVNNN
            ___1 TO 2240 OF 16B5CONTIG112701 (TRANSLATED) ____>

          370       380       390       400       410
AY366523_p  TLTSFERALSLAENEEEAADVWYNLGHVAVGIGDTNLAHQCFRLALVNNN>
            |||||||||||||||||||||||||||||||||||||||||||||||||||
Seq.No.5    TLTSFERALSLAENEEEAADVWYNLGHVAVGIGDTNLAHQCFRLALVNNN

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Seq.No.5    NHAEAYNNLAVLEMRKGHVEQARALLQTASSLAPHMYEPHFNFATISDKI
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Seq.No.5    GDLQRSYVAAQKSEAAFPDHVDTQHLIKQLRQHFAML
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TTCCAGGAGAAGTAACCCTGCTCTGTGGAATTGCAAGAATCTATGAGGAA

       160        170        180        190        200
ATGAACAATATGTCATCAGCAGCAGAATATTACAAAGAAGTTTTGAAACA

       210        220        230        240        250
AGACAATACTCATGTGGGAGCCATCGCATGCATTGGAAGCAACCACTTCT

       260        270        280        290        300
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       310        320        330        340        350
ATGGGCATTTATAACGGCCAGCTTTTTAACAATCTNGGGGCTGTGTTGCT

       360        370        380        390        400
TCTATGCCCAGCAGTATGATATGACTCTGACCTCATTTGAACGTGCCCTT

       410        420        430        440        450
TCTTTGGCTGAAAATGAAGAAGAGGCAGCTGATGTCTGGTACAACTTGGG

       460        470        480        490        500
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       560        570        580        590        600
GCTGTGCTGGAGATGCGGAAGGGCCACGTTGAACAGGCAAGGGCACTATT

       610        620        630        640        650
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       660        670        680        690        700
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       710        720        730        740        750
GCGCAGAAGTCTGAAGCAGCATTTCCAGACCATGTGGACACACAACATTT

       760        770        780        790        800
AATTAAACAATTAAGGCAGCATTTTGCTATGCTCTGATTGTTCCTTAGAC

       810        820        830        840        850
CACATATGTTCTTATGAAGCAGCATTATGCAAGGGGAAAAAAGCACTATG

       860        870        880        890        900
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       910        920        930        940        950
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       960        970        980        990       1000
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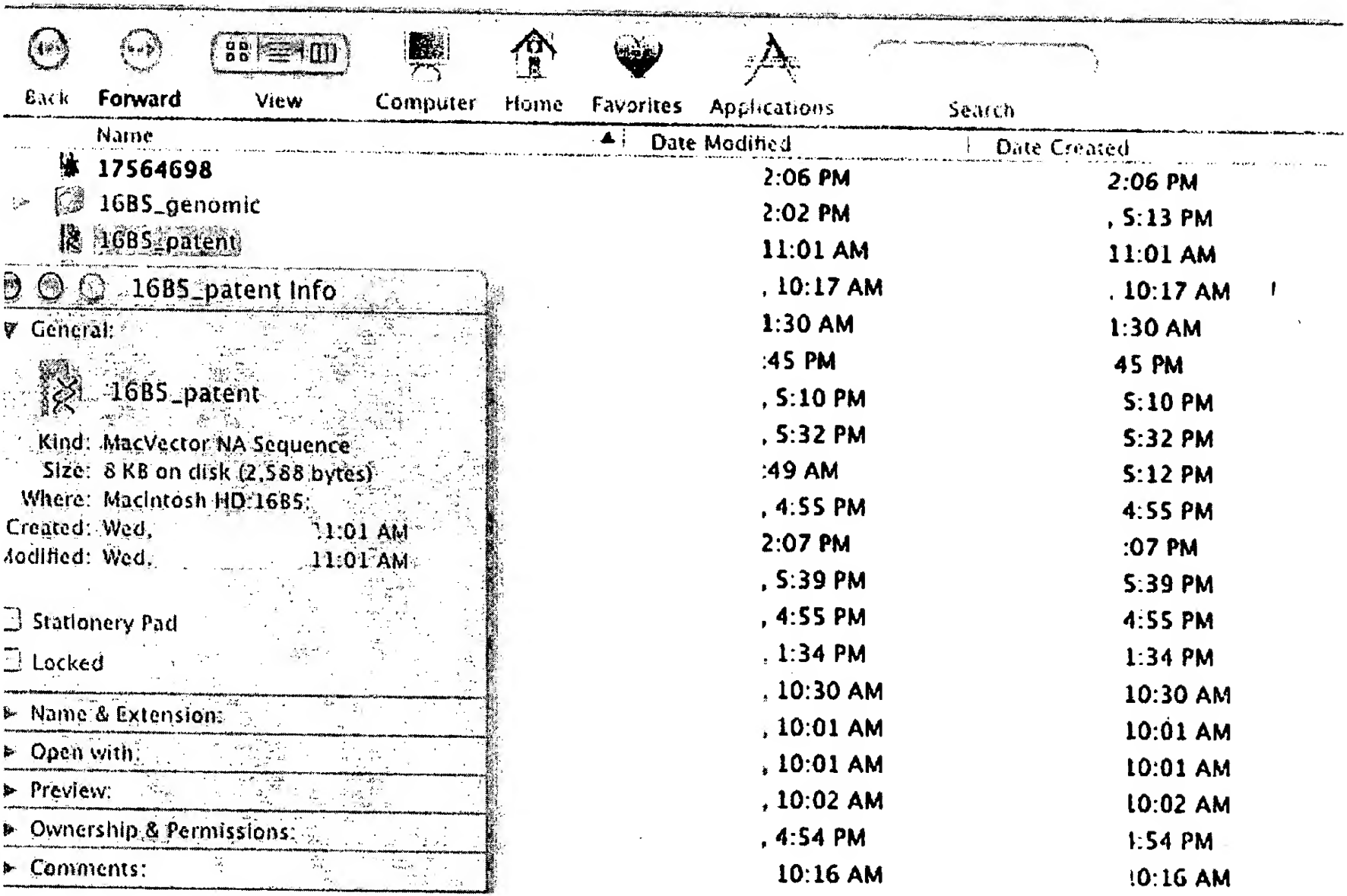
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Open with:
, 10:01 AM
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Preview:
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Comments:
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Sequence Range: 1 to 2240

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                60        70        80        90       100
ARP3       GAGGCTCTCCTGGAGCGCTGGGCCTTCGCTGGCCGCACCGGCAGCCATGA

               110       120       130       140       150
ARP3       GCTCGGAGATGGAGCCGCTGCTCCTGGCCTGGAGCTATTTTAGGCGCAGG

               160       170       180       190       200
ARP3       AAGTTCCAGCTCTGCGCCGATCTATGCACGCAGATGCTGGAGAAGTCCCC

               210       220       230       240       250
ARP3       TTATGACCAGGCAGCTTGGATCTTAAAAGCAAGAGCGCTAACAGAAATGG

               260       270       280       290       300
ARP3       TATACATAGATGAAATTGATGTAGATCAGGAAGGAATTGCAGAAATGATG

               310       320       330       340       350
ARP3       CTGGATGAAAATGCTATAGCTCAAGTTCCACGCCCTGGAACGTCTTTGAA

               360       370       380       390       400
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               410       420       430       440       450
ARP3       TCACACAAGCTGGAAGACCCATTACAGGTTTCCTCAGGCCCAGCACGCAG

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               560       570       580       590       600
ARP3       GAACGGCTTCCATGCTTACAAGTCCTGATGGACCATTTATAAATTTATCT

               610       620       630       640       650
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               660       670       680       690       700
ARP3       TTTGAGTATATCTTTCATCATGAAAATGATGTTAAGACTGCTTTGGATCT
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ARP3        GGCTGGCCCTCTCCACAGAACATTCTCAGTACAAGGACTGGTGGTGGAAA

                760       770       780       790       800
ARP3        GTACAGATTGGAAAATGTTACTACAGGTTGGGAATGTATCGTGAAGCAGA

                810       820       830       840       850
ARP3        AAAACAGTTTAAATCAGCCCTGAAGCAGCAGGAAATGGTAGATACATTTC

                                                 10        20
jmp1 str +                                  CAGGAAATGGTAGATACATTTC>
                                            ||||||||||||||||||||||
ARP3                                        CAGGAAATGGTAGATACATTTC

                860       870       880       890       900
ARP3        TGTACTTGGCAAAAGTTTATGTCTCATTGGATCAACCTGTGACTGCTTTA

               30        40        50        60        70
jmp1 str +  TGTACTTGGCAAAAGTTTATGTCTCATTGGATCAACCTGTGACTGCTTTA>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        TGTACTTGGCAAAAGTTTATGTCTCATTGGATCAACCTGTGACTGCTTTA

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            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        AATCTTTTCAAACAAGGCTTAGATAAGTTTCCAGGAGAAGTAACCCTGCT

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             130       140       150       160       170
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ARP3        CTGTGGAATTGCAAGAATCTATGAGGAAATGAACAATATGTCATCAGCAG

              1010      1020      1030      1040      1050
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ARP3        CAGAATATTACAAAGAAGTTTTGAAACAAGACAATACTCATGTGGAAGCC

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jmp1 str +  ATCGCATGCATTGGAAGCAACCACTTCTATTCTGATCAGCCAGAAATAGC>
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                         340       350       360       370
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jmp1 str +      TTTTTAACAATCTNGGG>
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ARP3            TTTTTAACAATCTGGGG


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                    380       390       400       410       420
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                      1310      1320      1330      1340      1350
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                    480       490       500       510       520
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                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            ATACAAATTTGGCCCATCAGTGCTTCAGGCTGGCTCTGGTCAACAACAAC


                      1360      1370      1380      1390      1400
ARP3            AACCACGCCGAGGCCTACAACAACCTGGCTGTGCTGGAGATGCGGAAGGG

                    530       540       550       560       570
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```
jmp1 str +  AACCACGCCGAGGCCTACAACAACCTGGCTGTGCTGGAGATGCGGAAGGG>
            ||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        AACCACGCCGAGGCCTACAACAACCTGGCTGTGCTGGAGATGCGGAAGGG


                  1410      1420      1430      1440      1450
ARP3        CCACGTTGAACAGGCAAGGGCACTATTACAAACTGCATCATCATTAGCAC

               580       590       600       610       620
jmp1 str +  CCACGTTGAACAGGCAAGGGCACTATTACAAACTGCATCATCATTAGCAC>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        CCACGTTGAACAGGCAAGGGCACTATTACAAACTGCATCATCATTAGCAC


                  1460      1470      1480      1490      1500
ARP3        CCCATATGTATGAACCGCATTTTAATTTTGCAACAATCTCTGATAAGATT

               630       640       650       660       670
jmp1 str +  CCCATATGTATGAACCGCATTTTAATTTTGCAACAATCTCTGATAAGATT>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        CCCATATGTATGAACCGCATTTTAATTTTGCAACAATCTCTGATAAGATT


                  1510      1520      1530      1540      1550
ARP3        GGAGATCTGCAGAGAAGCTATGTTGCTGCGCAGAAGTCTGAAGCAGCATT

               680       690       700       710       720
jmp1 str +  GGAGATCTGCAGAGAAGCTATGTTGCTGCGCAGAAGTCTGAAGCAGCATT>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        GGAGATCTGCAGAGAAGCTATGTTGCTGCGCAGAAGTCTGAAGCAGCATT


                  1560      1570      1580      1590      1600
ARP3        TCCAGACCATGTGGACACACAACATTTAATTAAACAATTAAGGCAGCATT

               730       740       750       760       770
jmp1 str +  TCCAGACCATGTGGACACACAACATTTAATTAAACAATTAAGGCAGCATT>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        TCCAGACCATGTGGACACACAACATTTAATTAAACAATTAAGGCAGCATT


                  1610      1620      1630      1640      1650
ARP3        TTGCTATGCTCTGATTGTTCCTTAGACCACATATGTTCTTATGAAGCAGC

               780       790       800       810       820
jmp1 str +  TTGCTATGCTCTGATTGTTCCTTAGACCACATATGTTCTTATGAAGCAGC>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        TTGCTATGCTCTGATTGTTCCTTAGACCACATATGTTCTTATGAAGCAGC


                  1660      1670      1680      1690      1700
ARP3        ATTATGCAAGGGGAAAAAAGCACTATGTCTGTGTATGTATGTATATAGTG

               830       840       850       860       870
jmp1 str +  ATTATGCAAGGGGAAAAAAGCACTATGTCTGTGTATGTATGTATATAGTG>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        ATTATGCAAGGGGAAAAAAGCACTATGTCTGTGTATGTATGTATATAGTG
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                1710      1720      1730      1740      1750
ARP3            TAATACGTATATTTTAACAAACCTGTCCTTGATATTAGTTAAGGTGACAC

                 880       890       900       910       920
jmp1 str +      TAATACGTATATTTTAACAAACCTGTCCTTGATATTAGTTAAGGTGACAC>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            TAATACGTATATTTTAACAAACCTGTCCTTGATATTAGTTAAGGTGACAC

                1760      1770      1780      1790      1800
ARP3            ATAAGGGTGACACAGAATGTGTAATGCAAATTTCATAGTAATAGTAACTT

                 930       940       950       960       970
jmp1 str +      ATAAGGGTGACACAGAATGTGTAATGCAAATTTCATAGTAATAGTAACTT>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            ATAAGGGTGACACAGAATGTGTAATGCAAATTTCATAGTAATAGTAACTT

                1810      1820      1830      1840      1850
ARP3            TATAAAATAATATTATAAAATACAGGATTTAAACCTTTCTAAATAGATCC

                 980       990      1000      1010      1020
jmp1 str +      TATAAAATAATATTATAAAATACAGGATTTAAACCTTTCTAAATAGATCC>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            TATAAAATAATATTATAAAATACAGGATTTAAACCTTTCTAAATAGATCC

                1860      1870      1880      1890      1900
ARP3            TGAAACTGTCTCTCACATTATATAGTAGATGTTTGTTTATAATGTTTACA

                1030      1040      1050      1060      1070
jmp1 str +      TGAAACTGTCTCTCACATTATATAGTAGATGTTTGTTTATAATGTTTACA>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            TGAAACTGTCTCTCACATTATATAGTAGATGTTTGTTTATAATGTTTACA

                1910      1920      1930      1940      1950
ARP3            AAACATTTTGGTGAATTTCCTCAATGTTTTATAAATGTACATTTTTTAAG

                1080      1090      1100      1110      1120
jmp1 str +      AAACATTTTGGTGAATTTCCTCAATGTTTTATAAATGTACATTTTTTAAG>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            AAACATTTTGGTGAATTTCCTCAATGTTTTATAAATGTACATTTTTTAAG

                1960      1970      1980      1990      2000
ARP3            TCCTTAAGCTGACTCTTAGCCATCATGTAGCTTAAGGAGTCTGAAATCTG

                1130      1140      1150      1160      1170
jmp1 str +      TCCTTAAGCTGACTCTTAGCCATCATGTAGCTTAAGGAGTCTGAAATCTG>
                ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3            TCCTTAAGCTGACTCTTAGCCATCATGTAGCTTAAGGAGTCTGAAATCTG

                2010      2020      2030      2040      2050
ARP3            CCATTAAAACTGCACCTTTAAGCCAGGTGTGGTAGCATGTGCCTATAGTC
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                1180      1190      1200      1210      1220
jmp1 str +  CCATTAAAACTGCACCTTTAAGCCAGGTGTGGTAGCATGTGCCTATAGTC>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        CCATTAAAACTGCACCTTTAAGCCAGGTGTGGTAGCATGTGCCTATAGTC


                     2060      2070      2080      2090      2100
ARP3        CCAGCTACTTGGGAGGTGGAGGTGGGAGGATTATAAATAGAGACTTTCCT

                1230      1240      1250      1260      1270
jmp1 str +  CCAGCTACTTGGGAGGTGGAGGTGGGAGGATTATAAATAGAGACTTTCCT>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        CCAGCTACTTGGGAGGTGGAGGTGGGAGGATTATAAATAGAGACTTTCCT


                     2110      2120      2130      2140      2150
ARP3        TAAGACTTTAAAAATGTATTTAAAACTATTTTTTATTAAATACTTTGTGA

                1280      1290      1300      1310      1320
jmp1 str +  TAAGACTTTAAAAATGTATTTAAAACTATTTTTTATTAAATACTTTGTGA>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        TAAGACTTTAAAAATGTATTTAAAACTATTTTTTATTAAATACTTTGTGA


                     2160      2170      2180      2190      2200
ARP3        TTTCCTATTAAGCTTTAAAATAAATCATTGTGTAAAACACCATCAAAGCG

                1330      1340      1350      1360      1370
jmp1 str +  TTTCCTATTAAGCTTTAAAATAAATCATTGTGTAAAACACCATCAAAGCG>
            ||||||||||||||||||||||||||||||||||||||||||||||||||
ARP3        TTTCCTATTAAGCTTTAAAATAAATCATTGTGTAAAACACCATCAAAGCG


                     2210      2220      2230      2240
ARP3        ATAAGCTCTGTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

                1380
jmp1 str +  ATAAGCTCTGTAA>
            |||||||||||||
ARP3        ATAAGCTCTGTAA
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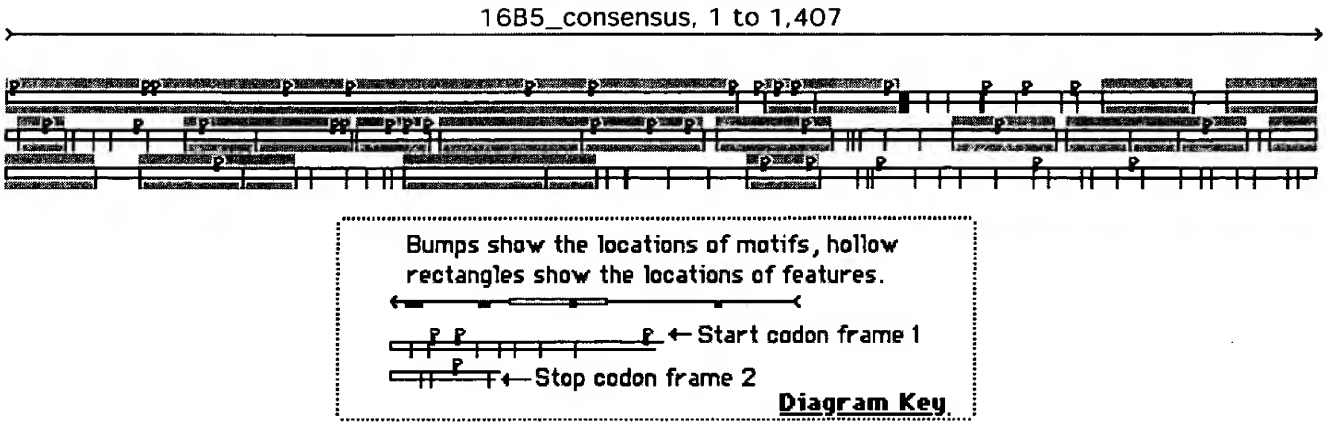
16B5_consensus
[Strand]



16B5_consensus
[Strand]

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41      ATGTCTCATT GGATCAACCT GTGACTGCTT TAAATCTTTT
81      CAAACAAGGC TTAGATAAGT TTCCAGGAGA AGTAACCCTG
121     CTCTGTGGAA TTGCAAGAAT CTATGAGGAA ATGAACAATA
161     TGTCATCAGC AGCAGAATAT TACAAAGAAG TTTTGAAACA
201     AGACAATACT CATGTGGAAG CCATCGCATG CATTGGAAGC
241     AACCACTTCT ATTCTGATCA GCCAGAAATA GCTCTCCGGT
281     TTTACAGGCG GCTGCTGCAG ATGGGCATTT ATAACGGCCA
321     GCTTTTTAAC AATCTGGGGC TGTGTTGCTT CTATGCCCAG
361     CAGTATGATA TGACTCTGAC CTCATTTGAA CGTGCCCTTT
401     CTTTGGCTGA AAATGAAGAA GAGGCAGCTG ATGTCTGGTA
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481     TTGGCCCATC AGTGCTTCAG GCTGGCTCTG GTCAACAACA
521     ACAACCACGC CGAGGCCTAC AACAACCTGG CTGTGCTGGA
561     GATGCGGAAG GGCCACGTTG AACAGGCAAG GGCACTATTA
601     CAAACTGCAT CATCATTAGC ACCCCATATG TATGAACCGC
641     ATTTTAATTT TGCAACAATC TCTGATAAGA TTGGAGATCT
681     GCAGAGAAGC TATGTTGCTG CGCAGAAGTC TGAAGCAGCA
721     TTTCCAGACC ATGTGGACAC ACAACATTTA ATTAAACAAT
761     TAAGGCAGCA TTTTGCTATG CTCTGATTGT TCCTTAGACC
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841     AGCACTATGT CTGTGTATGT ATGTATATAG TGTAATACGT
881     ATATTTTAAC AAACCTGTCC TTGATATTAG TTAAGGTGAC
921     ACATAAGGGT GACACAGAAT GTGTAATGCA AATTTCATAG
961     TAATAGTAAC TTTATAAAAT AATATTATAA AATACAGGAT
1001    TTAAACCTTT CTAAATAGAT CCTGAAACTG TCTCTCACAT
1041    TATATAGTAG ATGTTTGTTT ATAATGTTTA CAAAACATTT
1081    TGGTGAATTT CCTCAATGTT TTATAAATGT ACATTTTTTA
1121    AGTCCTTAAG CTGACTCTTA GCCATCATGT AGCTTAAGGA
1161    GTCTGAAATC TGCCATTAAA ACTGCACCTT TAAGCCAGGT
1201    GTGGTAGCAT GTGCCTATAG TCCCAGCTAC TTGGGAGGTG
1241    GAGGTGGGAG GATTATAAAT AGAGACTTTC CTTAAGACTT
1281    TAAAAATGTA TTTAAAACTA TTTTTTATTA AATACTTTGT
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1361    ACCATCAAAG CGATAAGCTC TGTAAAAAAA AAAAAAAAAA
1401    AAAAAAA
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